

Biological Confinement of Transgenic Plants

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Excision of selectable marker genes from transgenic plants

Peter D. Hare and Nam-Hoi Chua

Molecular strategies for gene containment in transgenic crops

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Table 1. Current and future technologies for transgene containment^a

Technique	Advantages	Disadvantages	Status
Maternal inheritance	Prevents gene flow through outcrossing and volunteer seeds. Relatively well developed. Field tests indicate low incidence of sympatry and mixed stands extinct in three years. High levels of transgene expression and no evidence for gene silencing or position effects.	Techniques to export proteins are not yet available. Foreign proteins have not been targeted to ER for glycosylation.	Demonstrated in tobacco, potato, and tomato. Further development required to extend to other food crops.
Male sterility	Prevents outcrossing. Shelf-life of flowers may also be extended. Several tapetum-specific promoters available.	Crop needs to be propagated by cross-pollination from non-GM crop or by artificial seeds. Potential for volunteer seed dispersal.	Demonstrated in tobacco and commercialized in glufosinate-tolerant rapeseed.
Seed sterility	Controls both outcrossing and volunteer seed dispersal.	If transgene is silenced, introgression will occur. All linked genes should segregate together.	Terminator technology has not been demonstrated in the field. RBF demonstrated in tobacco.
Cleistogamy	Pollination occurs before flower opens, theoretically preventing outcrossing.	Genes to modify floral design not readily available. In practice, introgression occurs despite self-pollination.	Not yet demonstrated in transgenic crops.
Apomixis	Seed is of vegetative origin and not from sexual cross. Controls both outcrossing and volunteer seed dispersal. Hybrid traits can be fixed.	Only known in a few crops. Genes not yet available.	Not yet demonstrated in transgenic crops.
Incompatible genomes	Prevents recombination after pollination.	May not be applicable to crops that exhibit homologous recombination. Crops will not produce seed unless propagated with compatible plants.	Not yet demonstrated in transgenic crops.
Temporal and tissue-specific control via inducible promoters	Gene either activated only when product is necessary or excised before flowering.	May not be applicable to traits required throughout the plant's life. If chemical treatment fails to penetrate plant tissues, residual levels of transgene may be present in pollen or seed that could be outcrossed.	Not yet demonstrated in transgenic crops.
Transgenic mitigation	Neutral for crops, but harmful for weeds.	Does not address gene flow between crops and may force wild relatives to extinction.	Not yet demonstrated in transgenic crops.

^aAbbreviations: ER, endoplasmic reticulum; RBF, recoverable block of function.

Bioconfinement Methods in Plants -- NRC Report			
Purpose	Method	Major Limitations	Other Considerations
Confine all gene flow via pollen and seeds	Sterile triploids or interspecific hybrids	Few triploid or sterile hybrid cases apply or are effective	Not useful if seed production is desired
	Use only male or only female plants that can be propagated vegetatively	Not feasible if same species or compatible relatives could cross-pollinate with unisexual plants; sex expression can be leaky	Not useful if seed production is desired
	V-GURT, such as original terminator	V-GURT under development (early); other sterility methods require vegetative propagation	V-GURT should not be used in food crops if growers need to save seeds
Reduce spread and persistence of vegetative propagules	V-GURT with inducible promoters that kill vegetative tissues	Under development (early)	
Confine pollen only	Male sterility	Available for some species, could be lost in later generations; transgenic methods could be more durable	Crop requires other plants as source of pollen if seed production is desired
	Transgene in chloroplast; Maternal inheritance	Under development; not feasible for plants with paternal inheritance of chloroplast DNA (most gymnosperms)	Possible to obtain high concentrations of desired genetically engineered proteins, but many traits cannot be conferred by chloroplast genes
	Cleistogamy (closed flowers)	Under development (early)	Results in self-pollination
	Apomixis (asexually produced seeds)	Under development (early)	Hybrid varieties would have high yield and breed true; could become invasive

NRC Report, Continued			
Purpose	Method	Major Limitations	Other Considerations
Transgenes absent in seeds and pollen	Transgenes only in rootstocks	Under development (early); cannot use transgenic traits in flowers, fruits, seeds	Applicable to grafted scions of certain woody species such as grapes, fruit trees
	Transgenes excised before reproduction	Under development (early); very speculative; cannot use transgenic traits in flowers, fruits, seeds	Allows seed production without spread of transgenes
Confine transgenic traits only (transgenes can spread)	T-GURT ^s involving inducible traits	Under development (early); external cues for transgene expression might not be reliable enough for high efficacy	Potentially useful; avoids concerns about sterile plants, but inactive transgenes can still spread
Reduce gene flow to and from crop relatives	Repressible seed lethality	Under development (early)	Allows viable seeds to be produced on same cultivar. Seeds sired on other cultivars or wild relatives would not be viable
	Cross-incompatibility	Under development (early); speculative	
	Chromosome location in allopolyploids	Under development; possible if relative has nonhomologous chromosomes; can be leaky	Applies only to crops that are allopolyploids (wheat, cotton, canola)
	Tandem constructs to reduce fitness in crop-wild hybrids and their progeny	Under development (early); requires fitness-reducing trait detrimental to wild plants but not crop	

NRC Report, Continued			
Purpose	Method	Major Limitations	Other Considerations
Phenotypic and fitness handicaps to reduce need for confinement	Domestication phenotypes	Under development; does not prevent gene flow	
	Auxotrophy (dependence on specific nutrients or growing conditions)	Under development; does not prevent gene flow	
Reduce exposure to transgenic products in plants	Tissue- and organ-specific promoters that limit expression of transgene	Promoters available, but greater efficacy needed in many cases; confines transgenic traits but not the transgenes; transgenes can spread	Could alleviate the need for bioconfinement in some cases
Minimize or eliminate need for bioconfinement	Choice of alternative organisms; choice not to release in field; choice not to proceed with GEO	Economic costs can be high, especially if decision to change course is made after economic investment	Often feasible and highly recommended when appropriate; alternative choices should be examined before GEO is developed

Seed Sterility: Terminator Technology

- Induces plants to produce non-viable offspring.
- Induction can occur by soaking seeds in a solution that induces a promoter.
- Uninduced seeds can develop into fertile plants – incomplete induction is a concern.
- Better to engineer sterility with an option for restoration of fertility (FAO 2002).
- **Weaknesses**
- The efficacy could be diminished by gene silencing, recombination
- Public access to data limited.

Recoverable Block of Function –

Kuvstimov et al, 2001

- Blocker _ DNA sequence element that interrupts a specific function resulting in death
- Recovery – DNA sequence restores blocked function activated by exogenous chemical treatment.
- Both the Blocker and Recovery sequences are physically linked to the transgene.
- Still in early stages of Development.

Repressive Seed lethal Confinement

- Novel trait is tightly linked to seed lethality in a hemizygous plants (SL/-)
- This is crossed with a homozygous plant containing the repressor (R/R).
- Seed with SL/R should express the novel trait, produce seeds.
- Sexually compatible relatives (-/-) should produce SL/- (non-viable) or R/- (only repressor).
- Similar to the terminator technology but facilitates seed production (25% not viable).

Repressive Seed lethal Confinement

Continued...

Weaknesses

- Not possible to cross with other useful cultivars.
- Early stages of development.
- Site specific integration of transgenes not yet achieved.
- Partial containment is possible as long as the linked transgenes are located on homologous chromosomes.
- Nearby related crops may produce dead seeds.
- Introgresssion of repressor genes into natural populations.
- Does not prevent seed mediated transgene escape

Unisexual plants lacking mates

- Examples: Holly, kiwi, ginkgo, avocado, asparagus
- Sex specific molecular markers can be use before massive propogation (Khadka et al, 2002)
- May be used in combination with other confinement approaches.

Weaknesses –

Applies to a narrow range of species

Dioecy is known to be quite leaky.

Seeds are produced by “male” plants (Poppendieck & Peterson, 1995).

Cleistogamy (closed flowers)

- Fertilization occurs before flower opens
- Obligate cleistogams would be effective in preventing gene escape via pollen

Weaknesses:

- Not yet available
- Perpetual self-fertilization could result in inbreeding, depression.
- Seed mediated dispersal not prevented

Apomixis (asexually produced seeds)

- Reproduce asexually by clonally produced seeds.
- Progeny are genetically identical to parent.
- Preserves superior genotypes (no need for inbred lines).
- Obligate apomixis is extremely rare.
- Most apomicts retain low to moderate sexual pollination to stimulate seed formation, in the absence of fertilization.
- Apomictic species easily outcompete sexual organisms (e.g. dandelions).

Weaknesses

- Apomictic GMOs could establish invasive populations.
- Not suitable for bioconfinement because even obligate apomicts produce seeds.

Transgenes absent from seeds and Pollen

Non-transgenic scions on transgenic rootstock

- Grapes, citrus, avocados are grown as grafted composites of two genotypes.
- Non-transgenic scions could be grafted onto transgenic rootstocks.
- Double-grafting (transgenic section sandwiched between non-transgenic) would prevent vegetative propagules.

Weaknesses

- Not useful for non-woody species.
- Applicable for traits in the root stock.
- Not applicable for forest trees (large scale).

Excision of transgenes before reproduction

- Trait expressed during vegetative growth.
- Does not result in seed sterility.
- A chemically induced flower specific promoter drives a recombinase enzyme that excised the transgene (Cre/lox system).

Weaknesses

- Extremely difficult to guarantee reliability.
- Not applicable for traits in seeds.

Artificially induced transgenes expression

- Trait is activated by a chemical spray
- Example: Salicylic acid for pathogen resistance
- An example of T-GURT.

Weaknesses

- Bioconfinement of expression but not transgene
- Early stages of development
- Not applicable to traits that require constant expression.

Cross- incompatibility

- Crosses between incompatible species fail entirely or fail most often.
- Alleles for incompatibility could offer new bioconfinement strategies.
- Not yet available
- Does not prevent seed mediated dispersal.

Chromosomal locations in Allopolyploids

- Wheat, coffee, peanut, and allopolyploids
- Wheat (AA, BB, DD) and goat grass share “D” genome.
- Therefore transgenes inserted into A or B genome will not introgress into wild populations.
- Oil seed rape (AACC) shares the A set of chromosomes with the weed B. campestris. Therefore transgenes integrated into the ‘C’ chromosomes will be excluded in the wild (Gressel 1999).

Weaknesses

- Decrease in the frequency of transgenic plants within the first back-cross can be explained by selection against the A chromosome (Tomiuk et al, 2000).
- This technique would not necessarily limit transmission of transgenes into the F2 progeny of crop-wild hybrids

Fitness reduction in Transgenic Crop-wild progeny

- Transgenic mitigation may be achieved by linking both sides of a transgene with traits neutral for crop but deleterious for weeds.
- Deleterious traits include lack of seed dormancy, seed shattering, dwarfing or susceptibility to herbicide.
- Was demonstrated with herbicide resistance and dwarfing (Ahmad and Gressel, 200).
- Dwarfed plants were competitively inferior.

Weaknesses

- Serious concern on endangered plant species.
- Tight link of TM alleles I necessary.
- Nearby related crops may produce unfit plants.

Reducing exposure to transgenic traits

- Tissue specific expression might reduce environmental exposure.
- Chloroplast targeting: expression free of transgenic products in seeds, pollen, roots. (not really!!).
- Roots and tuber specific: potatoes, carrots, (patatin, carotenoid specific promoters), root nodule specific expression.
- Vascular tissue specific: ideal to control aphids/hoppers. Phloem specific promoters available for rice and oat.
- Flower and fruit specific: promoters are available for sepals or carotenoid rich tissues (fruit).
- Pollen specific: Allergic asthma effect of rye grass pollen was reduced using pollen specific antisense technology.
- Seed specific: Barley aleuron specific promoters, soybean, bean seed specific promoters are available.

Reducing exposure to transgenic traits

continued...

Weaknesses

- More studies are needed to understand tissue/organ specific gene expression.
- Bioconfinement will not be improved, although exposure to gene product may be reduced.

Mortality of Vegetative Propagules

- Serious concern in semi-domesticated and non-domesticated grasses, trees, shrubs.
- Programmed cell death and Hypersensitive response to pathogens releases a signal that induce senescence.
- The signaling pathway involves changes in the antioxidant systems that are activated by nitric oxide and reactive oxygen. (DePinto et al, 2002)

Mortality of Vegetative Propagules

Continued...

- At MYB 30 transcriptional regulation gene, a positive regulator of hypersensitive cell death program (Vaillean et al, 2002).
- Lethal leaf spot 1 (Lls 1) suppresses cell death.
- It is possible to engineer environmentally triggered programmed cell death.
- Gene silencing, recombination or incomplete induction may complicate engineering, plants that destruct reliably at a given time.

Male Sterility

Sterile food crops: Banana, seedless grapes

Interspecific hybrids:

- Often not complete male sterile
- Eg. sorghum bicolor x S.halepense hybrids are similar to parents in tiller number, seed set, pollen viability and biomass
- Hybrids reproduce vigorously by vegetative reproduction than fertile relatives

Male Sterility, continued

- Strength – When triploid hybrids maintain sterility, genes are unlikely to spread via pollen or seed.
- Weaknesses – This will not be a general solution for transgene containment because seeds are often needed as end products. Also, interspecific hybrids offer moderate bioconfinement or none at all in some cases.

Non-transgenic male sterility

- Used in hybrid seed production eg. Sunflower, sorghum, canola,
- Genic – Mutation in nuclear genes, dominant – not useful.
- Cytoplasmic – mitochondrial genome rearrangement.
- CMS- Nuclear restorer genes.
- CMS – opportunity for reversion is a disadvantage.
- Male sterility is recommended to control transgenes flow

Sterile Triploids

- Contain 3 sets of chromosomes
- Results from a cross between diploid and tetraploid species
- Triploids are partially or fully sterile
- Maintained through vegetative propagation
- Possible option for bioconfinement

Weaknesses

Efficacy of triploid induction varies by genotype and environment

Transgenic Sterility - Nonreversible

- Useful for clonally propagated plants e.g. poplar
- Ablate floral tissues by expression of cytokinin in a tissue specific manner (Strauss et al, 1995).

Weaknesses

- Requirement for vegetative propagation.
- long term sterility may require suppression of multiple genes or mechanisms.
- - precludes options for further breeding and seed production
- - takes 5-10 years to test concept in trees or other perennials.
- Evolutionary dead-end.

Transgenic Male Sterility

Nuclear male sterility has been engineered in tobacco, rice, maize, alfalfa, Brassica by using the barnase gene (encodes a ribonuclease).

If expression is leaky, other tissues may be affected, but dual component system may overcome this (Burgess et al, 2002).

- Restoration of Barnase by barstar gene offered biocontainment and option for breeding in Indian oil seed mustard (Jagannath et al, 2002).

Transgenic Male Sterility

Continued...

Weaknesses

- Gene silencing, recombination may revert to fertility
- Cross-pollination is needed for seed set
- Pollen from weeds could pose problems
- Potential for seed dispersal exits.

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graph TD; A([Advantages of Chloroplast Transformation]) --- B((Hyper-expression)); A --- C((Multigene Engineering)); A --- D((Maternal Inheritance)); A --- E((Gene Containment)); A --- F((No Pleiotropic Effects)); A --- G((No Position Effect)); A --- H((No Gene Silencing)); A --- I((No Vector Sequences));
```

Advantages of Chloroplast Transformation

Hyper-expression

Multigene Engineering

Maternal Inheritance

Gene Containment

No Pleiotropic Effects

No Position Effect

No Gene Silencing

No Vector Sequences

Milestones in chloroplast genetic engineering: an environmentally friendly era in biotechnology

Henry Daniell, Muhammad S. Khan and Lori Allison

Multigene engineering: dawn of an exciting new era in biotechnology

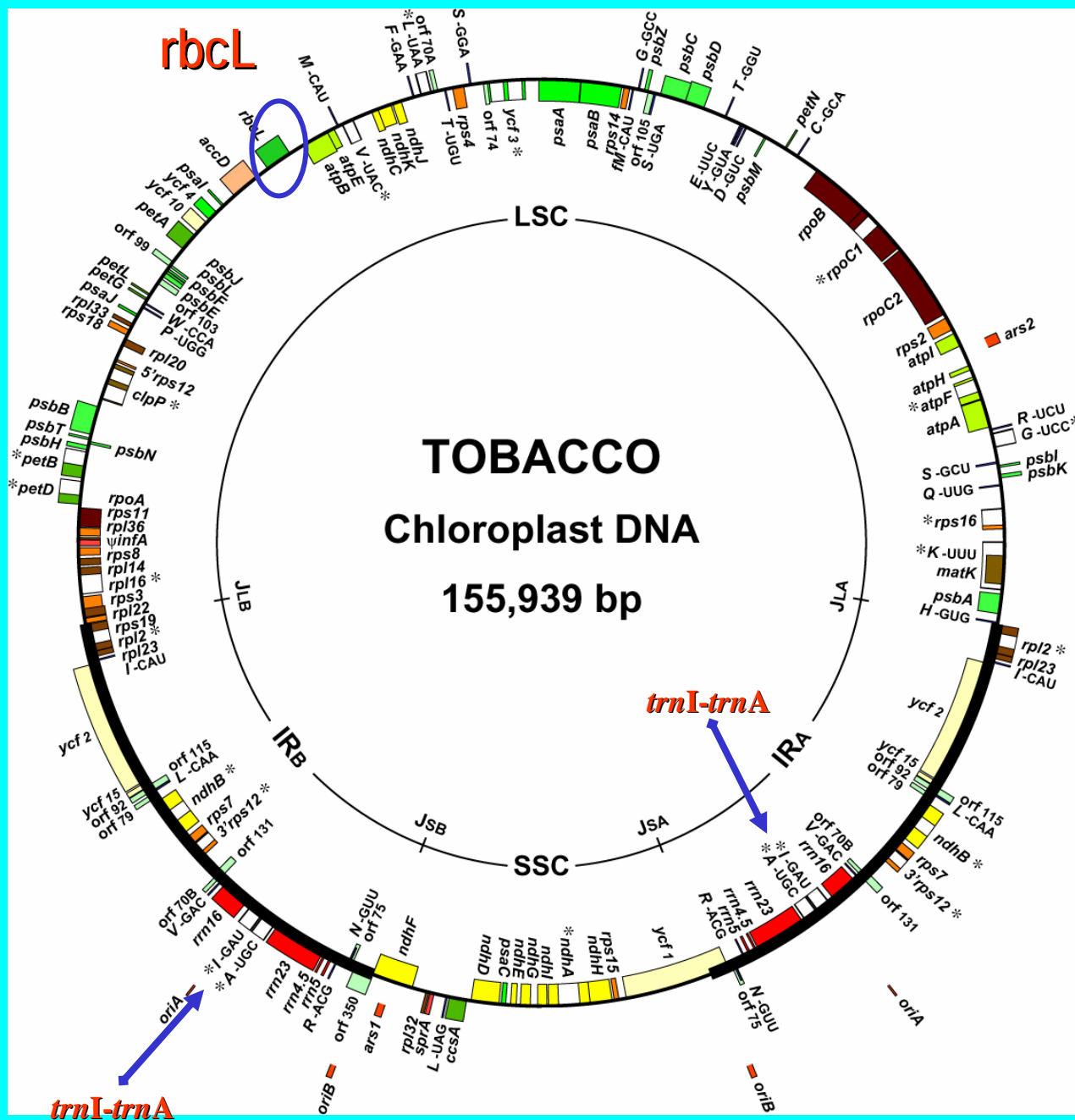
Henry Daniell* and Amit Dhingra

Current Opinion in Biotechnology 2002, 13:136–141

0958-1669/02/\$ – see front matter

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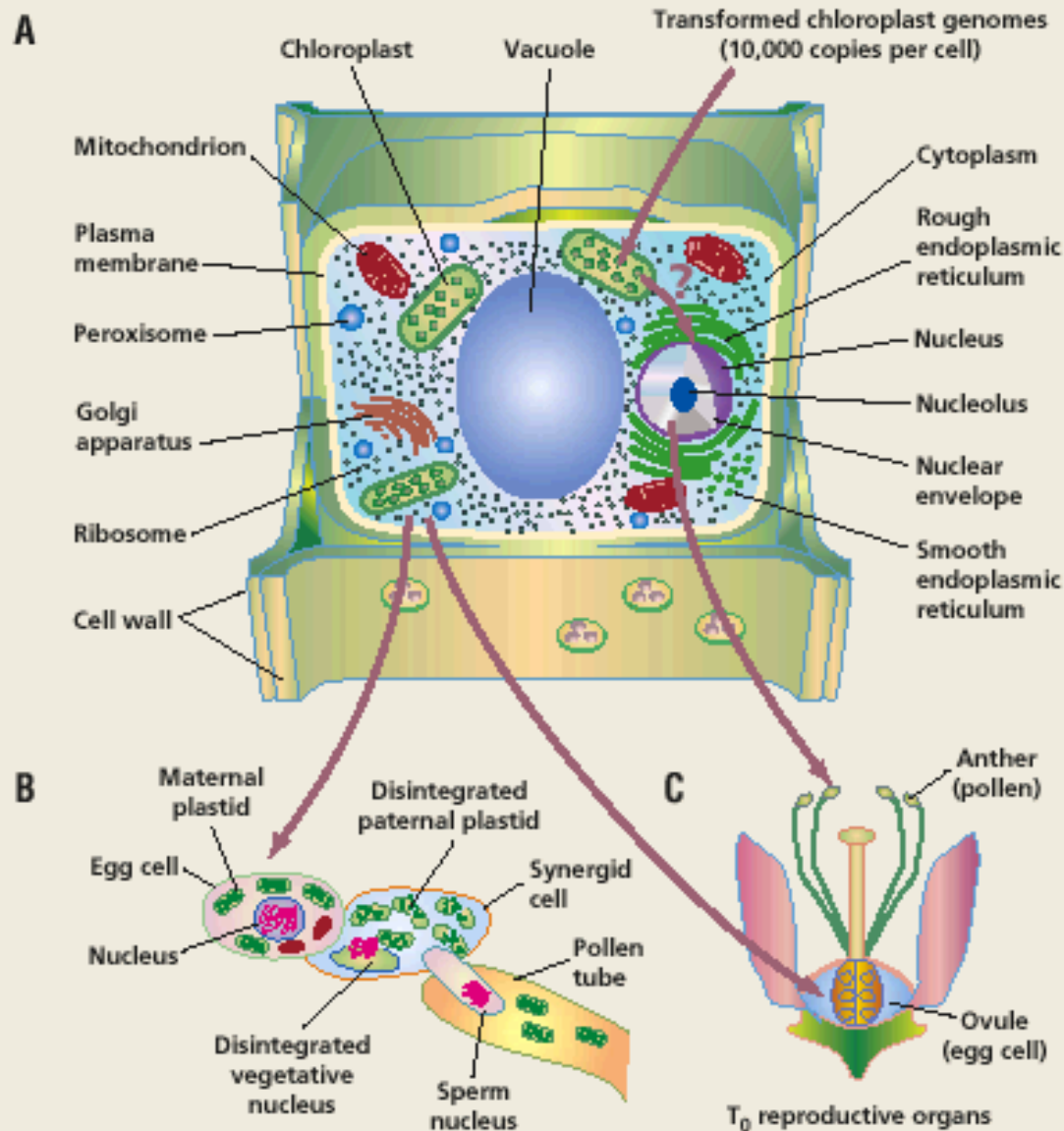


Figure 1. Maternal inheritance and gene containment. (A) The plant cell shows three compartments that contain DNA: nuclear, chloroplast, and mitochondrial genomes. The question mark raises the possibility that transgenes can jump from the chloroplast to nuclear genome. (B) During meiosis, haploid egg and sperm cells are formed. The synergid cell attracts the pollen tube by secretion of calcium, carbohydrates, and proteins. Fertilization begins when the pollen tube enters the synergid cell. Once inside the cytoplasm of the synergid cell, the pollen tube ruptures releasing its contents. The paternal chloroplasts are disintegrated and only the sperm nucleus enters the egg cell and fuses with the egg to form zygote. The zygote contains only maternal plastids because the paternal plastids disintegrate in the synergid cell. Thus, maternal inheritance of transgenes offers containment because of lack of gene flow through pollen. (C) Reproductive floral organs. Anthers produce pollen. Ovules contain egg cells.



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Grants Program

*Daniell, H., R. Datta, S. Varma,
S. Gray and S.B. Lee. 1998.
Containment of herbicide resis-
tance through genetic engineer-
ing of the chloroplast genome.
Nature Biotechnology 16: 345-
348.*



eed control often re-
quires the use of herbi-
cides that also may
negatively affect crop
plants. Glyphosate (i.e.,

Roundup) is a common herbicide that
kills both grasses/sedges and broad-
leaf plants by blocking the biochemical
pathway producing essential amino

acids (phenylalanine, tyrosine, and tryptophan). Only plants, fungi, and bacteria can make these essential amino acids. Animals (including humans) are insensitive to glyphosate, making its use relatively safe. Plant resistance to glyphosate has already been genetically engineered using genetic material in the cell nucleus. However, there is now concern over the use of such plants because the resistant genes could be spread with the release of pollen. This release could lead to a decrease in the overall effectiveness of the herbicide against weeds and create "superweeds." Daniell and colleagues, with the support of NRI funding, have found a solution to this problem by using genetic material in the chloroplast to genetically engineer glyphosate-resistant tobacco. Chloroplast genetic material is maternally inherited and cannot be spread by pollen in most crops (with rare exceptions like pines). This chloroplast-derived resistance is also more resistant to glyphosate than the nuclear-derived resistance. Application of glyphosate after crop emergence is now possible without fear of uncontrolled spread of the resistance gene or herbicide damage to the crop.

*This research was supported by a grant from the NRICGP, Non-Food Characterization/Process/
Product Research Program, Enhancing Value and Use of Agricultural and Forest Products Division*

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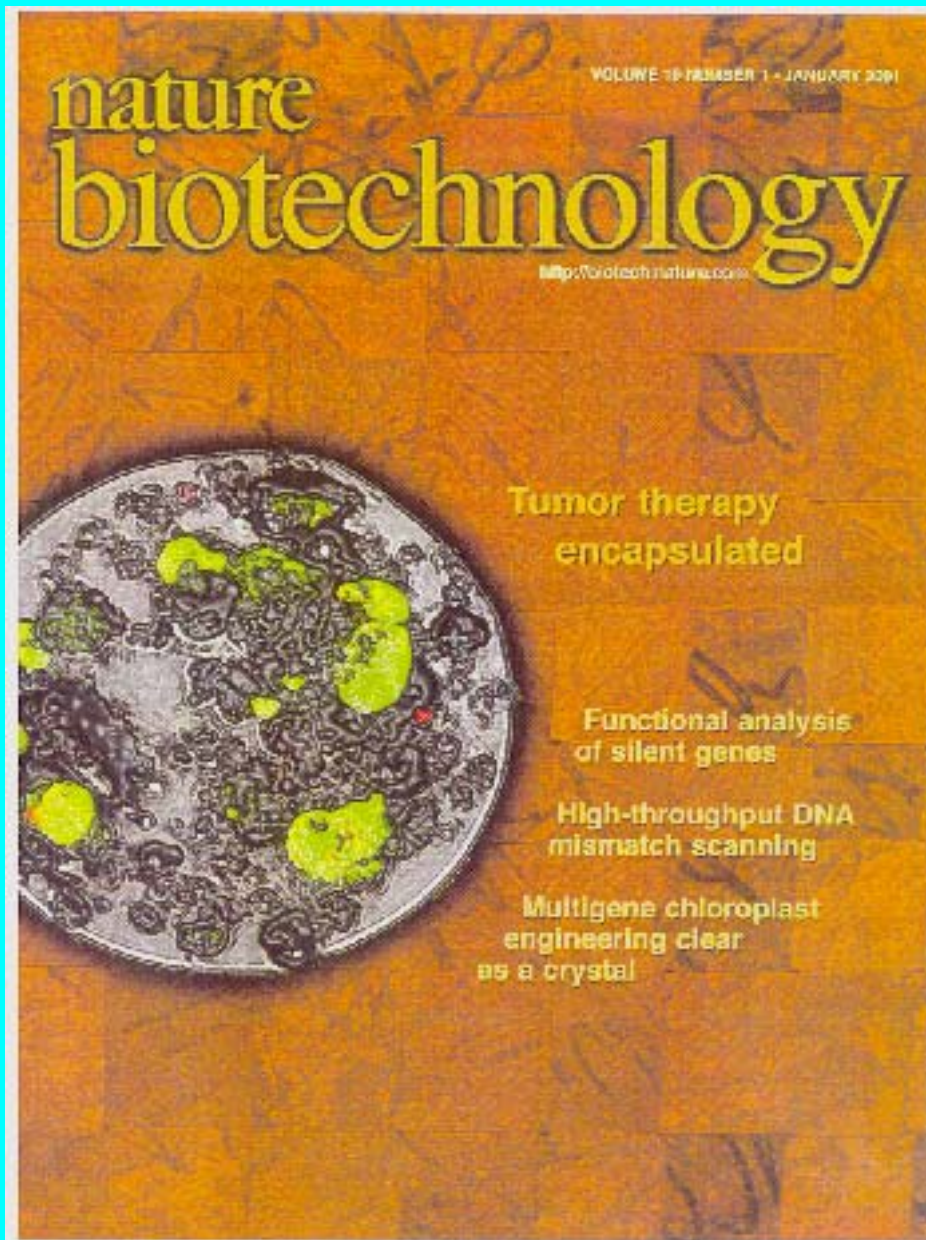
Bio-engineers find a way to 'contain' super plants

AUBURN, Alabama (CNN) -- Researchers at Auburn University have developed a technique that they say should wilt fears that genetically altered plants will spread their genes around. Based on experiments with tobacco plants, scientists say they can now confine certain implanted characteristics to a single species.

The basic problem that the Auburn scientists were tackling was this: While scientists have already been able to genetically engineer crops that are resistant to weeds and bugs, they want to also make sure that those "super powers" will not be transferred to nearby weeds -- a process that could make the weeds resistant and allow them to spread out of control.



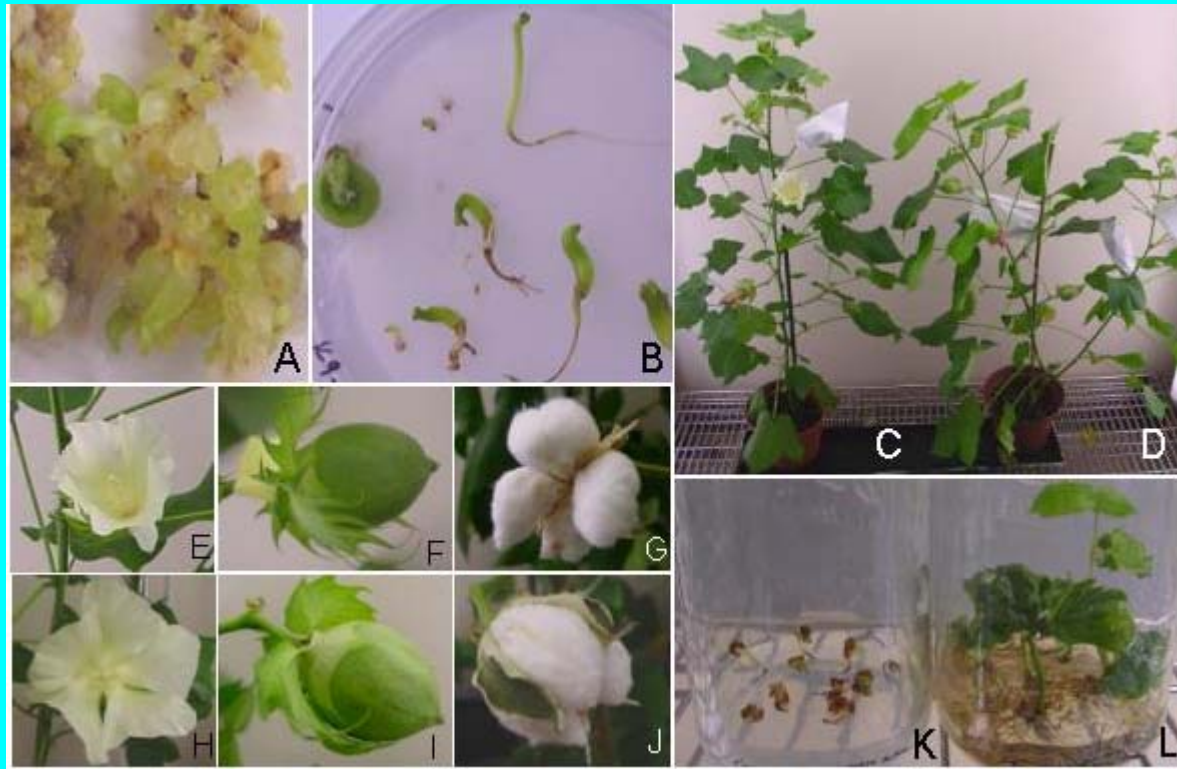
Auburn University researchers are creating bio-engineered tobacco plants that are herbicide resistant



**Overexpression of
the Bt cry2Aa2
operon in
chloroplasts leads
to formation of
insecticidal crystals**

**De Cosa B, Moar W, Lee SB,
Miller M, Daniell H
Nature Biotechnology (2001)
19:71-4**

Cotton Plastid Transformation



Daniell lab, Plant Molecular Biology, September 2004

Carrot Plastid Transformation



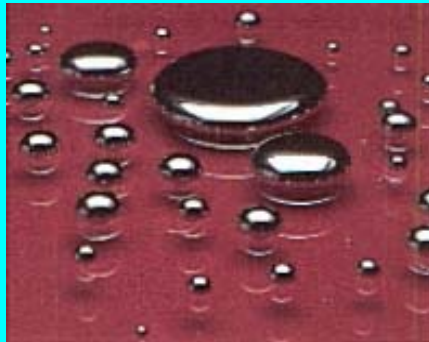
Daniell lab, Plant Physiology, September 2004

Soybean Plastid Transformation



Bayer Crop Science Lab, Plant Molecular
Biology, August 2004

Phytoremediation of organomercurial compounds via Chloroplast genetic Genome



¹Oscar N. Ruiz, ²Hussein Mohamed, ²Norman Terry, ¹Henry Daniell

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Plant Physiology 132: 1-9, July 2003

Accumulation of Trehalose within Transgenic Chloroplasts Confers Drought Tolerance

**SB Lee, M Byun and H Daniell
Molecular Breeding 11: 1-13
January 2003.**

Expression of an Antimicrobial Peptide via the Chloroplast Genome to Control Phytopathogenic Bacteria and Fungi

**DeGray G, Rajasekaran K, Smith F, Sanford J, Daniell H
Plant Physiology (2001) 127:852-62**

Nominated for best paper of 2001

AGRONOMIC TRAITS EXPRESSED VIA CHLOROPLAST GENETIC ENGINEERING

AGRONOMIC TRAITS	GENE	SPACER REGION	PROMOTER	5'/3' REGULATORY ELEMENTS	LAB
Salt tolerance (Carrot)	<i>badh</i>	<i>trnI/trnA</i>	Prrn-F	ggagg/rps16	Daniell
Insect resistance	<i>Cry2Aa2</i> Operon	<i>trnI/trnA</i>	Prrn	Native 5'UTRs / <i>TpsbA</i>	Daniell
Disease resistance	<i>MSI-99</i>	<i>trnI/trnA</i>	Prrn	ggagg / <i>TpsbA</i>	Daniell
Drought tolerance	<i>Tps</i>	<i>trnI/trnA</i>	Prrn	ggagg / <i>TpsbA</i>	Daniell
Phytoremediation	<i>merA^a/merB^b</i>	<i>trnI/trnA</i>	Prrn	ggagg ^{a, b} / <i>TpsbA</i>	Daniell
Herbicide resistance	<i>aroA</i> (petunia)	<i>trnI/trnA, rbcL/accD</i>	Prrn	ggagg / <i>TpsbA</i>	Daniell
Insect resistance	<i>Cry2Aa2</i>	<i>rbcL/accD</i>	Prrn	ggagg (native) / <i>TpsbA</i>	Daniell
Herbicide resistance	<i>bar</i>	<i>rbcL/accD</i>	Prrn	<i>rbcL</i> / <i>TpsbA</i>	Day
Insect resistance	<i>Cry1A(c)</i>	<i>trnV/rps12/7</i>	Prrn	<i>rbcL</i> / <i>Trps16</i>	McBride

Tobacco -vs- *E. coli*

*1 tobacco plant produces a million seeds.

*The leaves of 1 tobacco plant produce more recombinant protein than a 300-liter fermenter in *E. coli* (Crop Tech, VA)

*It costs about 50x's more to produce 1kg of a recombinant protein in *E. coli* than in transgenic plants (Petridis et al, 1995)



Manipulation of Gene Regulation in Transgenic Chloroplasts Results in Hyper-expression of Human Serum Albumin, Formation of Inclusion Bodies and Facilitates Purification

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**Plant Biotechnology Journal 1: 71-79
(March 2003)**

Recombinant Human Serum Albumin

- The world's most used intravenous protein
- Current need for 500 metric tons per year
- Average dose per day 20-40 grams(~\$8/gm)
\$700 million current U.S. market - growing
- Current need for tissue culture and formulation over 100 metric tons annually
- Removes risk of exposure to human viruses

Expression of Interferon $\alpha 2b$ in Transgenic Chloroplasts of a Low Nicotine Tobacco

Daniell et al., Vaccine, October 2004

Expression of Guy's 13 in Transgenic Chloroplasts

DENTAL CARIES



Different stages of development

Guy's 13 assembly in transgenic chloroplasts

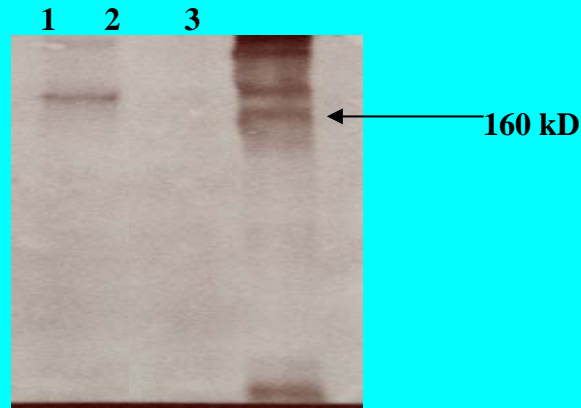


Figure 7: Western Blot Analysis of transgenic lines showing the assembled antibody.

Lane 1 : Extract from a transgenic line, Lane 2: Negative control-extract from an untransformed plant. Lane 3: Positive control-human IgA.

The gel was run under non-reducing conditions. The blot was developed with AP-conjugated goat anti-human kappa antibody.

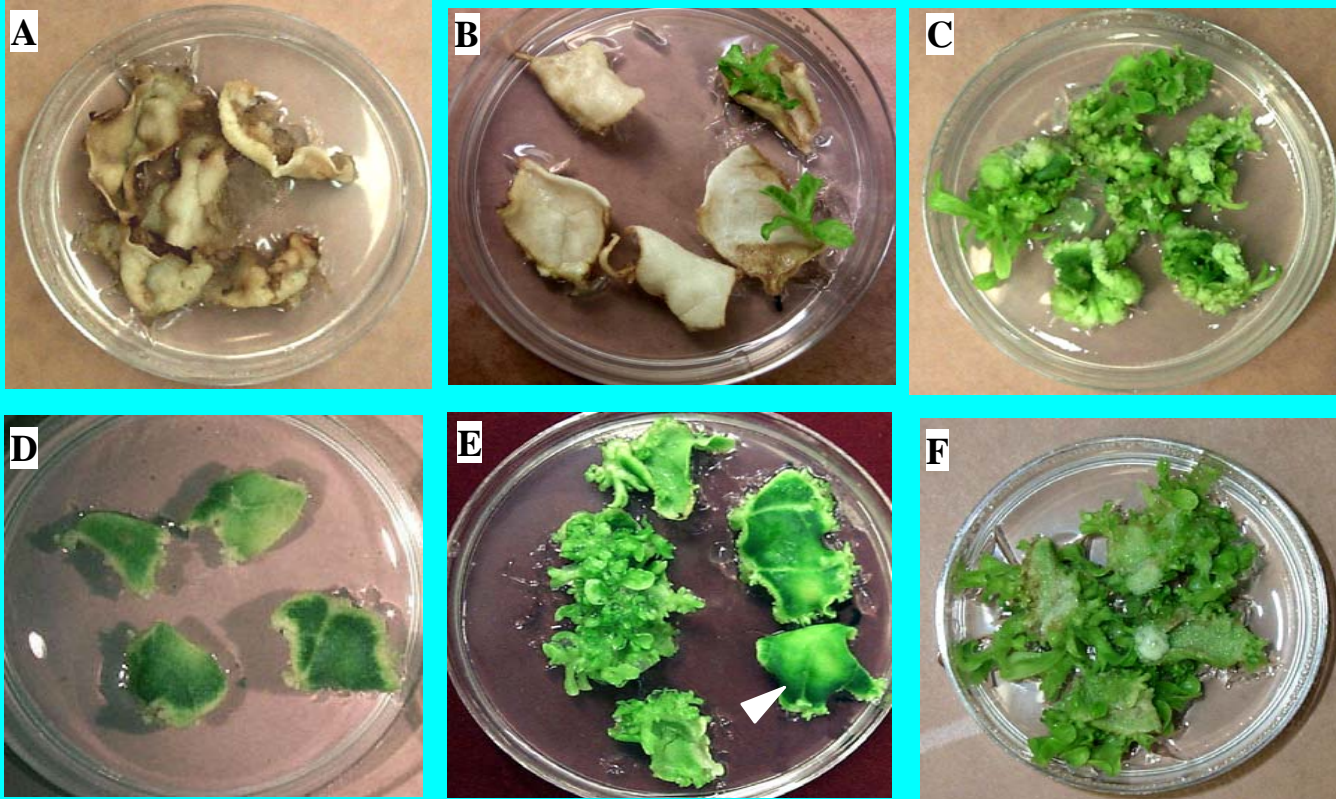
**Expression of *Bacillus anthracis*
Protective Antigen in
Transgenic Chloroplasts
Towards the Development of an
Improved Anthrax Vaccine**

Daniell et al., Vaccine (September 2004)

Chloroplast derived Anthrax Vaccine

- Up to 2.5mg PA /g fresh weight
- 172 mg PA per plant (Petit Havana)
- 400 million vaccine doses/acre (with 50% loss during purification)
- 18.17 fold increase in commercial cultivar in the field
- Current dosage is 1.75 to 7 ug, Eight doses are required for immunity
- Current vaccine is in limited supply and is contaminated with Lethal and Edema factor
 - Post-exposure vaccination with a regimen of antibiotics is also recommended

Marker free transgenic plants: engineering the chloroplast genome without the use of antibiotic selection.



Henry Daniell, B Muthukumar, Seung Bum Lee
Current Genetics (2001) 39(2):109-16

VACCINE ANTIGENS EXPRESSED VIA CHLOROPLAST GENETIC ENGINEERING

VACCINE ANTIGENS	GENE	SITE OF INTEGRATION	PROMOTER	5'/3' REGULATORY ELEMENTS	%TSP EXPRESSION	FUNCTIONALITY ASSAY	LAB
Cholera toxin	<i>ctxB</i>	<i>trnI/trnA</i>	Prrn	ggagg/ <i>TpsbA</i>	4%	GM-1 ganglioside binding assay	Daniell
Canine Parvovirus (CPV)	<i>ctxB</i> -2L21 <i>gfp</i> -2L21	<i>trnI/trnA</i>	Prrn	<i>PpsbA/TpsbA</i>	31.1%, 22.6%	Immunogenecity was demonstrated	Daniell/ Veramandi
Anthrax protective antigen	<i>pag</i>	<i>trnI/trnA</i>	Prrn	<i>PpsbA/TpsbA</i>	4 -5%	Macrophage lysis assay	Daniell
Plague vaccine	<i>caF1~LcrV</i>	<i>trnI/trnA</i>	Prrn	<i>PpsbA/TpsbA</i>	14.8%	ND	Daniell
Tetanus toxin	<i>tetC</i> (bacterial and synthetic)	<i>trnV/rps</i> 12/7	Prrn	T7 gene 10 ^a , <i>atpB</i> ^b / <i>TrbcL</i>	25% ^a , 10% ^b	Pathogen challenge and immunogenecity	Maliga

BIOPHARMACEUTICAL PROTEINS EXPRESSED VIA CHLOROPLAST GENETIC ENGINEERING

BIOPHARMA- CEUTICAL PROTEINS	GENE	SITE OF INTEGRATION	PROMO- TER	5'3' REGULATORY ELEMENTS	% TSP EXPRESSION	LAB
Elastin-derived polymer	EG121	<i>trnI/trnA</i>	Prrn	T7gene10 / <i>TpsbA</i>	ND	Daniell
Antimicrobial peptide	MSI-99	<i>trnI/trnA</i>	Prrn	ggagg / <i>TpsbA</i>	21.5%- 47%	Daniell
Insulin-like growth factor	<i>IGF-1</i>	<i>trnI/trnA</i>	Prrn	<i>PpsbA/TpsbA</i>	33%	Daniell
Interferon alpha 5	<i>INFα5</i>	<i>trnI/trnA</i>	Prrn	<i>PpsbA/TpsbA</i>	ND	Daniell
Interferon alpha 2b	<i>INFα2B</i>	<i>trnI/trnA</i>	Prrn	<i>PpsbA/TpsbA</i>	19%	Daniell
Human Serum Albumin	<i>hsa</i>	<i>trnI/trnA</i>	Prrn ^a , <i>PpsbA</i> ^b	ggagg ^a , <i>psbA</i> ^b / <i>TpsbA</i>	0.02% ^a , 11.1% ^b	Daniell
Monoclonal antibodies	<i>Guy's 13</i>	<i>trnI/trnA</i>	Prrn	ggagg/ <i>TpsbA</i>	ND	Daniell
Interferon gamma	<i>IFN-g</i>	<i>rbcL/accD</i>	<i>PpsbA</i>	<i>PpsbA/TpsbA</i>	6%	Reddy
Human somatotropin	<i>hST</i>	<i>trnV/rps12/7</i>	Prrn ^a , <i>PpsbA</i> ^b	T7gene10 ^a <i>psbA</i> ^b / <i>Trps16</i>	7.0 % ^a 1.0% ^b	Monsanto

Transgenes in Chloroplast DNA

Continued...

Weaknesses

- Biparental inheritance in gymnosperms
- Rye, kiwi chloroplast genome is paternally inherited.
- Alfaalfa – biparental inheritance
- Not yet available for cereals or monocots
- Seed mediated dispersal not prevented.

Dreams
made real

